V. Epidemiologic Study Design: Matched Cohort

A. Design Considerations

The goal of this study clearly mandates a comprehensive epidemiologic approach, incorporating mortality, and historical, current, and followup morbidity studies. Exposure to herbicides during the 1962-1971 time period may have initiated long-term health effects that may or may not be progressive. If such effects are detectable by a review of the subject's past medical history, and can be verified, direct links to compensation issues can be made. Current health status, as mirrored by a large number of recent VA claims and inquiries, is of major interest, because such claims and inquiries may indicate medical conditions that might be confirmed by a comprehensive physical examination. If analyses of both mortality and morbidity data yield only indeterminant or weakly suggestive findings, it may be that sufficient time has not yet passed for substantial emergence of longterm health effects. This dictates a requirement for a follow-up element to the study.

Methodological shortcomings are inherent in each element of this comprehensive study. To some extent, the classical deficiencies of each particular epidemiologic approach are compensated by the concurrent use of the other elements. For example, the low chance of identifying a relatively uncommon disease solely by the use of a mortality study is offset by the inclusion of a current morbidity study. The relatively quick feedback that can be attained from current morbidity and mortality studies will serve to better define the follow-up study, and will help to alleviate problems that arise as a result of changes in diagnostic criteria and methods over time. Nevertheless, problems that can affect ascertainment of disease in all phases of the study will remain. Inaccurate patient recall of antecedent events, the distortion of information by knowledge of anticipated symptomatology, and participant or observer knowledge of their exposure status can only be corrected to a limited extent by review of records for symptom validation and "blind" assessment protocols. In addition, fundamental problems dealing with adequate selection of a control group and limiting loss to study can influence any comprehensive epidemiologic investigation. These and other pitfalls in study design will be discussed in more detail in Section VIII.

The management of this project will be conducted through standard Air Force Research and Development procedures, including program monitors at Air Force Headquarters and Air Force Systems Command, and a Program Management office at Brooks AFB, Texas. Contract monitors will insure that all contractual efforts are conducted according to strict quality assurance procedures, and an on-site monitor will insure that the physical examinations are conducted in strict accordance with the study protocol.

Since the study has three elements and confronts a health issue with incompletely specified or uncertain endpoints, strong potential bias, and severe time contraints, the following design represents the best overall framework for achieving validity. The design process is complex and in itself time dependent.

B. Selection and Ascertainment of the Populations for Study

(1) The Exposed Military Groups

(a) Operation RANCH HAND Personnel

Operation RANCH HAND personnel flew C-123 aircraft in RVN during 1962-1971. Data from hand-compiled lists obtained through the RANCH HAND Association (a reunion organization), Air Force personnel records, unit historical records, and actual C-123 flight orders, place the herbicide exposed population at approximately 1264 individuals. Of those personnel confirmed as RANCH HAND participants, 25% are still on active or reserve duty, with the remainder being composed of retired, separated, or deceased persons. To identify all RANCH HAND participants, an indepth search was conducted of all organizational records stored at the Military Records Division, National Personnel Records Center (NPRC), St. Louis, Missouri.

Introductory letters will be sent to the last known address of all identified persons, and nonresponse will be pursued by cross-locator systems available within the government (e.g., Social Security Administration, VA, Internal Revenue Service). Significant efforts will be made to account for at least 99% of the total population (see Figure A-2, Section XV). Because of the limited number of RANCH HAND personnel, no subsampling of the exposed group is planned in any phase of the study. All members will be strongly encouraged to participate in all phases of the investigation.

All RANCH HAND personnel are males currently ranging in age from 30-69 years (mean = 42.4 years). The normal C-123 crew composition was one pilot, one copilot/navigator (both officers), and one spray equipment console operator (enlisted) in the rear of the aircraft. The aircrew officer-enlisted ratio is 2.2:1; however, the inclusion of RANCH HAND support personnel (predominantly enlisted) in the study will make the overall officer-enlisted ratio 1:1.7. Approximately 98% of the officers and 92% of the enlisted men were Caucasian. Attempts have been made to identify all maintenance personnel assigned to the RANCH HAND units. Maintenance of the RANCH HAND aircraft was performed within a step-wise organizational structure. Routine daily maintenance (primary) was conducted by flight line support personnel who were often dedicated exclusively to RANCH HAND operations. More extensive maintenance (secondary) was carried out by consolidated support units at the base level, which were also responsible for non-RANCH HAND C-123s as well. Major aircraft overhauls and modification were conducted by maintenance units at Clark Air Base, Philippines. The maintenance personnel in these centralized units were not directly assigned to RANCH HAND, and their exposures to RANCH HAND C-123 aircraft and herbicide cannot be validated. From 1962 through 1964, the primary flight line maintenance teams were dedicated to RANCH HAND aircraft, and these individuals have been identified by the mechanisms described above. In 1965, flight line maintenance was performed by personnel of the centralized maintenance organization (secondary), and it is not feasible to adequately identify all of these individuals from available records. After 1966, the RANCH HAND organization transferred their

base of operations to a new location, and primary maintenance was once again performed by personnel assigned specifically to RANCH HAND. These individuals have been readily identified. Thus, maintenance personnel directly assigned to RANCH HAND will be included in the study. These complexities are summarized in Table 4.

Table 4

FEASIBILITY OF IDENTIFYING AIRCRAFT MAINTENANCE PERSONNEL (TOTAL POPULATION) EXPOSED TO HERBICIDE ORANGE

Time	Primary Maint Personnel[1]	Secondary Maint Personnel[2]
Jan 1962-Jul 1964	Yes	No
Aug 1964-Dec 1966	Yes/No[3]	No
Jan 1967-Oct 1971	Yes	No

[1]individual assigned to RH; total number (denominator) known
[2]individual not assigned specifically to RH, although may have serviced the aircraft; denominator not ascertainable
[3]other documents permit ascertainment of a portion of this group

Because of the significant combat hazard associated with low, slow flying missions, some early RANCH HAND crewmembers were elite volunteers (see Risk-Taking Bias, Section VIII, C). In fact, RANCH HAND crewmembers comprised one of the most highly decorated units during the RVN Conflict. Anecdotal stories reveal that most crew members were, on occasion, heavily exposed to Herbicide Orange due to normal or combat induced equipment malfunctions within the aircraft. Many former RANCH HAND personnel are expected to be currently employed in the aerospace industry as commercial airline pilots, airline managers, and flight mechanics. RANCH HAND personnel still on active duty are expected to be found in senior management positions.

(b) Alternate Exposed Populations

(1) Introduction

The principal investigators, members of all of the peer review committees, and independent consultants have clearly recognized that the statistical power of this RANCH HAND study is suboptimal for the detection of specific uncommon conditions or diseases. This limitation is inherent because the size of the RANCH HAND population is fixed at approximately 1200 individuals, and it cannot be increased.

A brief review of alternate military populations is in order to highlight the significant advantages of the RANCH HAND population. The desire to achieve more optimal statistical power by merely increasing the size of the population under study must be balanced with a careful analytic process which assesses the exposure level of alternate populations, and categorizes them as either additive or nonadditive to the RANCH HAND study population.

(2) U.S. Army Ground Personnel

Some U.S. Army personnel were undoubtedly exposed to herbicides during their duty in Vietnam; however, the objective ascertainment of exposed individuals is not possible. Any attempts to identify individuals assigned to combat units which may have been exposed would result in an unacceptable degree of misclassification since U.S. Army personnel records do not exist which would allow the accurate identification of soldiers below the battalion level. This lack of demoninator data, and the high degree of misclassification in determining the exposure status of Army troops makes this population unsuitable for inclusion in the framework of the RANCH HAND Study.

(3) Ancillary Air Force Groups (Non-RANCH HAND Personnel)

Air Force handlers of herbicide drums in RVN were exposed to herbicides because of drum leakage. As the drum handlers were ad lib participants, no personnel designator was assigned to these individuals, thus prohibiting computer tracking and identification. The size of this population is unknown, but it is expected to be small (less than 200), as the majority of drum handlers are known to have been Vietnamese. Additional groups such as U.S. Army helicopter crews, casual observers (both Army and Air Force), and experimental fighter-bomber personnel who may have occasionally conducted spray operations were also potentially exposed. However, population-at-risk determinations for all of these groups cannot be made, and any identification of individuals exposed in these situations must rely on self-selection or incomplete ascertainment. Also, the selection of suitable control groups for a study of these individuals is difficult if not impossible.

(4) U.S. Marine Corps Troops

On 16 November 1979, the GAO released a report which suggested that a herbicide-exposed population of nearly 22,000 U.S. Marine Corps troops could be identified, and that this identified group would be appropriate to study. Records exist which locate Marine Corps battalion headquarters near the C-123 spray paths. The GAO made several improper assumptions to conclude that all of the identified marines were in fact exposed. Specifically, all battalion troops were assumed to be located at the battalion headquarters. Further, the effect of prevailing winds on the direction of spray drift, and the photodegradation of the chemicals were not considered by the GAO. The National Research Council panel considered the GAO analysis, and proposed a study of 5900 marines who were "near" spray paths on the same day

as the spraying. The "exposed" group was to be contrasted with the mortality experience of 212,000 presumably unexposed controls (also marines). The RANCH HAND study described in this protocol consists of approximately 1200 exposed individuals and 6000 controls for the mortality study phase. Despite the fact that the RANCH HAND Study involves a smaller sample size than the proposed Marine effort, the RANCH HAND Study is more powerful statistically. Specifically, lower exposure to herbicide by a conservative factor of from 1/10 to 1/1000 and misclassification in Marine exposure groups renders the Marine Study far less powerful than the RANCH HAND effort. As described in Section VI, misclassification and decreased exposure are seen to be independent factors additively decrementing Marine Study statistical power. Even when all 21,900 marines within the herbicide spray paths up to 28 days following the spray operations are considered exposed, the RANCH HAND Study is noted to be significantly superior.

(5) Conclusions

The Operation RANCH HAND participants are the most suitable of the military populations to study in evaluating the longterm effects of herbicide/dioxin exposures. The RANCH HAND group had a much higher level of exposure which was sustained over a prolonged period of time. This increased level of exposure implies that RANCH HAND personnel would be more likely to develop more acute and chronic symptoms from the exposure, and would manifest them sooner than the other exposed military personnel. The addition of significantly less exposed and/or misclassified groups to the RANCH HAND population for the attractive purpose of increasing statistical power would constitute an egregious dilutional error.

(2) Control Group (Not exposed to Herbicide Orange)

A review of all specialized flight units present in Southeast Asia during the RVN conflict, reveals clearly that there is no absolutely ideal control group for the RANCH HAND population. C-130 aircrew members and support personnel were selected because of sufficient population size, similar training profiles, and psychologic similarities to the RANCH HAND group.

Total ascertainment of the C-130 population is being conducted by computer and hand selection for specific military flying organizations, and foreign country service, during the interval from 1962 thru 1970. Over 2.3 million personnel records have been reviewed, and the approximate C-130 population size is 23,978 individuals. Aircrew members who flew C-130 aircraft in Southeast Asia during 1962-1970 were selected as controls for the RANCH HAND aircrew population. The C-130 flight line maintenance population were ascertained from personnel records by similar mechanisms, and served as the specific control population for the RANCH HAND support personnel. The proportions on active duty, and non-active duty status are expected to parallel the patterns in the exposed group.

Another possible control group, the non-RANCH HAND C-123 population, is known to be too small (approximately 3000) to provide adequate sampling flexibility and replacement under the proposed matched variable concept

(see below and Section VI). Also, many of the RANCH HAND aircraft were reconfigured for transport and insecticide missions and thus, the non-RANCH HAND C-123 crews responsible for these other missions may have been exposed to significant Herbicide Orange residue in these aircraft. Therefore, this group may not have been truly unexposed to herbicides, and was discarded as an appropriate control population. Crewmembers of C-7 transport aircraft were also considered as a potential control group; however, because of small sample size (1000-2000) and the fact that they served in RVN only during the post 1967 era, they were also dropped from consideration.

The normal crew composition of a C-130 is three officers and two enlisted personnel. The control group is considered to be "pure" from the standpoint of lack of occupational exposure to herbicide. The entire control group will be considered "nonvolunteer" with respect to abnormally high combat risk. While in general they will possess lifestyle characteristics and socio-economic backgrounds similar to the exposed group, their overall combat morbidity/mortality and the resultant stress influences upon general health may be slightly less than in the exposed group. For those separated and retired C-130 controls, similar proportions to the exposed group are expected to be employed in the aerospace industry. Known and estimated factors of the control and exposed populations are summarized in Table 5.

(3) Matching Procedures and Rationale

Each member of the exposed group has been computer matched to a set of C-130 controls comprised of approximately 10 individuals using three variables. Since the two groups are highly selected and inherently similar with respect to many variables, very close matches are feasible. This epidemiologic design incorporates a matched concept because: (1) a matched cohort design will provide maximum test power throughout the entire study, and (2) statistical intergroup comparisons may be made without normalization by three key variables known to effect symptom frequencies of interest, thus providing greater power for complex statistical testing. It is apparent that following the match, both exposed and control populations will be very nearly identical with respect to the three influencing variables so that a replacement concept is feasible (see F below). In the event that frequent match breaks occur, stratification techniques can be used.

The selection of the control group produces an inherent match for equivalent SEA experience, and additional matching has been conducted for (1) age, by year of birth and closest month possible, (2) Air Force Speciality Code (AFSC) as an absolute match, and (3) race (Caucasian versus non-Caucasian) as an absolute match. Specific rationale for these variables is as follows: (1) the age match controls for the many clinical symptoms and signs associated with advancing age, (2) AFSC controls for officer-enlisted status (as well as crewmember-noncrewmember status), a variable strongly linked to educational background, current socio-economic status, and moderately linked to age (5 year median difference) and socio-economic background, and (3) race controls for differences in chronic disease development, socio-economic background, etc.

Table 5

COMPARISON OF THE STUDY GROUP TO POSSIBLE CONTROL GROUPS BY KNOWN AND ESTIMATED FACTORS

KNOWN FACTORS	STUDY GROUP	POSSIBLE CONTROL GROUPS		
	RANCH HAND C-123	Non-RANCH HAND C-123	C-7	C-130
POPULATION SIZE	1264	3000	1200	23,978
OFFICER/ENLISTED RATIO	1:1.7	1:2	1:2	1:2
AIRCRAFT FUEL (AV-GAS)	YES (+JP-4)*	YES (+JP-4)*	YES	NO (JP-4 only)
OCCUPATIONAL HERBICIDE EXPOSURE	YES	YES/NO**	NO	NO
ESTIMATED FACTORS				
OCCUPATIONAL INSECTICIDE EXPOSURE	2+	0	0	0
COMBAT HAZARD	4+	3+	3+	2+
RVN-IN COUNTRY ASSIGNMENT	4+	4+	4+	2+

*In 1968, aircraft were modified with a JP-4 booster.
**Contaminated aircraft reconfigured for transport may have resulted in exposure to non-RANCH HAND personnel.

The inherent match for SEA experience controls for combat-induced physiologic, psychophysiologic, and other related morbidity and mortality disorders. Additionally, this inherent match may reflect the effects of alcohol consumption, the use of chemoprophylactic and/or illicit drugs, and the acquisition of tropical diseases associated with life in SEA. The comparisons of the exposed (RANCH HAND) subjects and their selected sets of controls are detailed in Appendix Table A-4. Only 4 of the ten categorical AFSC/case strata had less than ten controls for each exposed subject. The group of Caucasian pilots had a mean of only 9.5 controls per exposed subject, due to the extreme ages of several individuals, and the strata of Black pilots and other Black officers had means of 2.7 and 5.0 controls respectively. However, since there were only seven black officers in the exposed group and only thirty controls, high numbers of tight matches could not be achieved. Black enlisted aircrewmembers had a mean of 9.8 controls each.

(4) Computer Science and Statistical Details of the Matching Process

As described above, the matching for this project has been performed using three variables: occupational category, race and age. Five occupational categories (officer/pilot, officer/navigator, officer/other, enlisted/flight engineer, and enlisted/other) have been used to reflect socioeconomic status and aeronautical rating. The variable of race has been dichotomized into black and non-black. Ten matched controls have been selected for each exposed subject, regardless of current vital status. The computer method applied to select the control subjects is an adaptation of a procedure studied by Raynor and Kupper (Nearest Neighbor Matching on a Continuous Variable, Technical Report, Department of Biostatistics, University of North Carolina, 1979). As the first step, the RANCH HAND and control groups were partitioned into ten strata using the categorical occupational and race variables. The Raynor and Kupper matching procedure was then applied iteratively within each of the strata to match for the continuous variable of age, given in months. The Raynor-Kupper procedure involves the following steps:

STEP #1. The RANCH HAND cohort in a given strata is randomly permuted.

STEP #2. The first RANCH HAND subject in the permuted set is selected for matching.

STEP #3. The closest available control is assigned to the selected RANCH HAND subject using the absolute value of the difference between the months of birth of the RANCH HAND and the control subjects. If the closest available control is further than 60 months from the selected RANCH HAND subject, a blank is assigned. Tied assignments are broken randomly.

STEP #4. Step #3 is repeated for all RANCH HAND subjects in the strata proceeding down through the permuted set, until the entire RANCH HAND cohort is exhausted.

STEP #5. Steps #1 through #4 are repeated ten times for each RANCH HAND subject to construct a 1:10 study set. At the completion of the matching activity, the RANCH HAND - Control study matrices for each of the ten occupation-race strata can be diagrammatically represented as in Figure #1.

Figure 1. MORTALITY ANALYSIS COHORTS

RANCH HAND COHORT	C_1 ——— CONTROL COHORTS ——— C_{10}					
R_1	$C_{1,1}$	$C_{1,2}$	$C_{1,3}$	$C_{1,3}$ ·····	$C_{1,10}$	
R_2	$C_{2,1}$	$C_{2,2}$	$C_{2,3}$	$C_{2,4}$ ·····	$C_{2,10}$	
R_3	$C_{3,1}$	$C_{3,2}$	$C_{3,3}$	$C_{3,4}$ ·····	$C_{3,10}$	
R_4	$C_{4,1}$	$C_{4,2}$	$C_{4,3}$	$C_{4,4}$ ·····	$C_{4,10}$	
.	.	.	.	.	.	
.	.	.	.	.	.	
.	.	.	.	.	.	
R_j	$C_{1200,1}$	$C_{1200,2}$	$C_{1200,3}$	$C_{1200,4}$·····		$C_{1200,10}$

Figure 2. MORTALITY MATRIX

RANCH HAND COHORT	C_1 ——— CONTROL COHORTS ——— C_{10}						
R_1	$C_{1,1,m'}$	$C_{1,2,m'}$	···	$C_{1,5,m'}$	$C_{1,6'}$	···	$C_{1,10'}$
R_j	$C_{j,1,m'}$	$C_{j,2,m'}$	···	$C_{j,5,m'}$	$C_{j,6'}$	···	$C_{j,10'}$
.	.	.		.	.		.
.	.	.		.	.		.
R_{1200}	$C_{1200,1,m'}$	$C_{1200,2,m'}$	···	$C_{1200,5,m'}$	$C_{1200,6'}$	···	$C_{1200,10'}$

In each row of this matrix the controls are ordered from nearest to farthest in terms of age of the matched RANCH HAND person. The next operation defining the control group involved randomization of all of the controls in each row of each stratum matrix to negate the ordering by age. Then, the first five members of each control set for each RANCH HAND person are identified as being subjects in the mortality portion of the study. The resulting occupation-race strata matrices now have the form shown in Figure 2.

In Figure 2, $C_{j,k'}$ or $C_{j,k,m'}$ may be equivalent to any $C_{j,k}$ of Figure 1 due to the randomization process.

Table 6 summarizes the results of the matching process, and Appendix Table A-5 provides a more complete statistical description of the process. In these tables, the age difference between the month of birth of the control and the month of birth of the RANCH HAND person, (counting months from 1900) and the cumulative number of controls and the cumulative percentage with this difference are shown.

Table 6. RESULTS OF THE MATCHING PROCESS (1:10)

Age Difference (in Months)	Cumulative Number of Controls	Cumulative Percent
0	8612	70.6
1	10287	84.3
2	10749	88.1
3	10984	90.1
4	11167	91.6
5	11322	92.8
6	11410	93.5
12	11688	95.8
24	11921	97.7
36	12028	98.6
48	12129	99.4
60	12197	100.0

(5) Study Group Selection Procedures

(a) Mortality Analysis

A 50% random sample of each control set will be drawn and used to comprise a 1:5 mortality analysis, as described in section (4). The vital status of each subject in this sample and of all exposed subjects will be ascertained at a minimum frequency of every five years for the 20 year duration of the study. Those individuals dying of combat causes will be excluded from the mortality analysis as it is assumed that combat death is independent of herbicide effect. Further, the known differential combat death rate between the RANCH HAND and control groups can be attributed to the hazardous and unique nature of the RANCH HAND mission. Twenty-two RANCH HAND personnel (15 officers and 7 enlisted) died in combat. Medical record reviews will be accomplished to assess the illness experience of these individuals prior to combat mortality.

(b) Historical Morbidity Study

Retrospective or historical health data will be gathered on each exposed subject and from the first randomly selected mortality control from his set by questionnaire techniques. Living but noncompliant controls in

the historical morbidity study will be replaced by a compliant control selected from the control set. In order to avoid an information gap for data on deceased individuals, surrogate interviews will be obtained from the first order next-of-kin of exposed and control subjects dying of noncombat related causes between the date of their assignment to Southeast Asia and the initiation of this study. Since the validity and accuracy of surrogate derived data may not be equivalent to data obtained directly from living study subjects and their spouses, these data will be subsetted for analysis. All available medical records, (military, VA, and civilian) will be reviewed for all subjects selected for this morbidity analysis.

(c) Prospective Morbidity Study

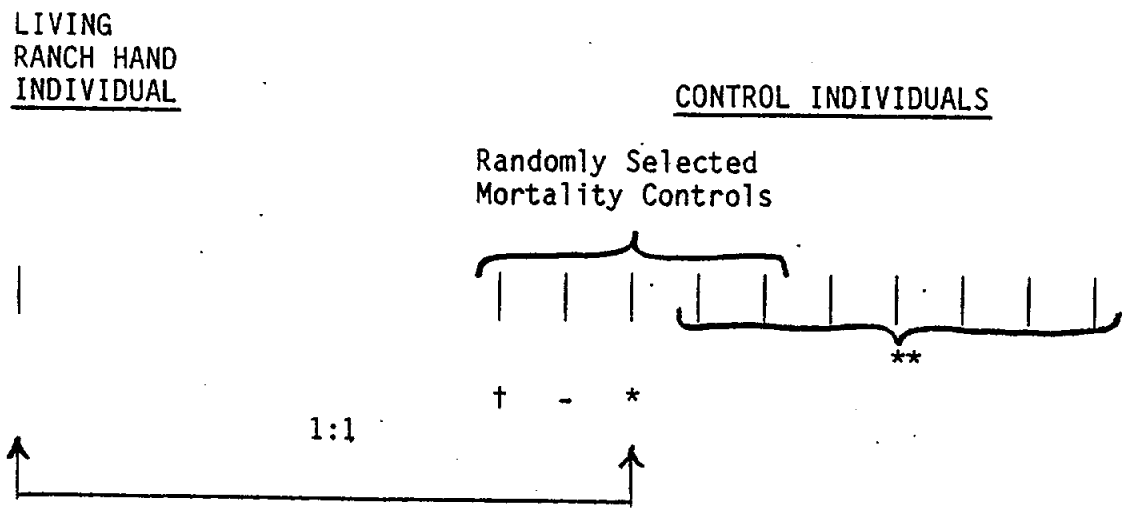
A baseline physical examination and review of systems will be conducted, and a prospective or followup approach will be used to assess the current state of health of study subjects using a series of questionnaires and physical examinations over the next 20 years. Each living exposed subject and the randomly selected primary control will be included in the questionnaire and physical examination phases. In this prospective study of morbidity, primary controls who are deceased, unaccountable or unwilling to participate in the followup studies, will be replaced by a willing subject from the remainder of the control set (Figure 3). The selected control for a RANCH HANDER dying of a noncombat cause will be retained throughout the questionnaire, physical examination, and followup phases of the study. Since the control's vital status and volunteerism should be independent of the matching sequence, many primary controls should enter the study. The remaining members of the control set will be used as replacement candidates for possible use later in the study (see section F below). All replacement controls will be clearly identified for the purposes of subset analysis so that population differences, if any, between the first randomly assigned selectees (noncompliant) and the replacements (compliant) can be assessed. Specific rules and procedures for study entry are found in Table A-6 and Figure A-3 of the Appendix.

(d) The Interrelatedness of the Comparison Groups

It should be clear from the foregoing discussion that the study populations of the mortality, historical morbidity, and prospective followup phases are highly related but different. Once selected, the mortality control cohorts will remain unchanged throughout the 20 years of observation. The population under study in the historical morbidity phase will initially be a randomly selected subset of the mortality comparison group; however, some of these primary controls may be decreased or noncompliant for the voluntary aspects of this phase of the study. In this phase, noncompliant controls will be replaced, but deceased controls will not, as surrogate interviews with the next-of-kin will be used to reconstruct morbidity data. The subsetting and replacement procedures create the difference between the mortality and historical morbidity comparison groups. The population in the prospective morbidity phase is the comparison group from the retrospective phase plus additional replacements for the deceased controls. Thus, it is clear that the comparison groups are slightly different, but they would be identical if no deaths occurred since 1962 and all primary controls were compliant.

Figure 3.

SELECTION PROCEDURE FOR THE QUESTIONNAIRE, PHYSICAL EXAMINATION, AND FOLLOW-UP STUDY

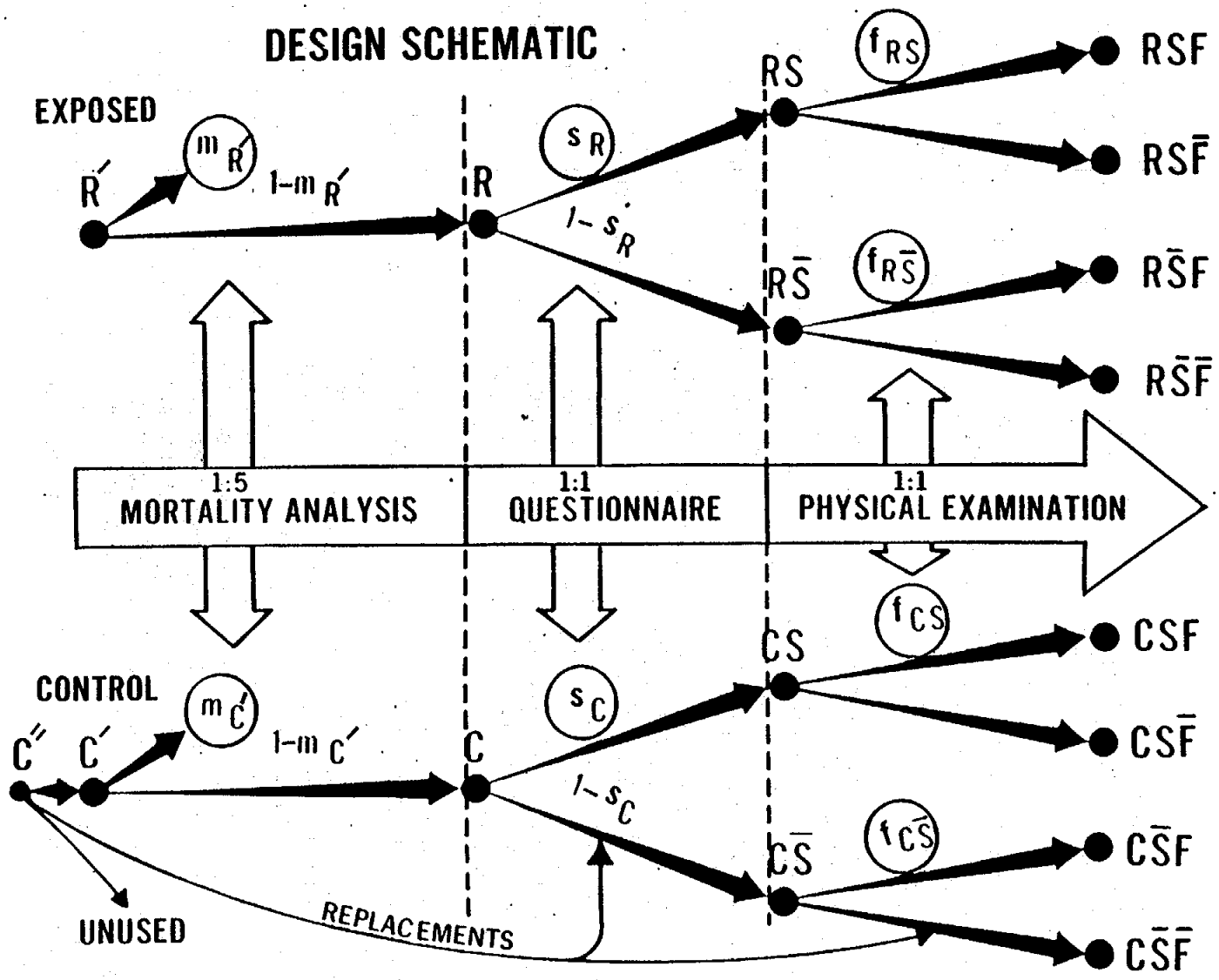


† Deceased
- Unwilling
* Volunteer
** Replacement Candidates

C. Overview of Statistical Methodology

The design of the study is presented in schematic form in Figure 4. R' refers to RANCH HAND personnel and C" refers to the collection of all possible control individuals. As defined, R' and C" will contain individuals who are deceased of noncombat causes. Combat deaths are excluded from R' and C". Since C" is approximately 20 times larger than R', a randomized subsample C' and C" will be obtained. C' will be constructed from C" by computer selection of the ten matched controls for each exposed study subject. As previously noted, close matches will be made for the variables of age, AFSC, and race. The matched controls will form ten cohorts, C_1 through C_{10}, as shown in Figure 1. A 50% random sample from each of the matched control sets of 10 will be selected for inclusion in the mortality assessment so that a group, C' is obtained that consists of 5 matched controls for each exposed subject. These controls will be designated as initial replacement candidates for the morbidity and follow-up studies. The remaining individuals in the control set will be additional replacement candidates in the event that replacement must occur beyond the members of the mortality set (see Figure 3). C' will be constructed without regard to whether the individual is currently living or dead so that an assessment of noncombat mortality can be accomplished.

FIGURE 4


DESIGN SCHEMATIC
EXPOSED
$m_{R'}$
$1-m_{R'}$
R'
R
s_R
$1-s_R$
RS
f_{RS}
RSF
RS̄F̄
RS̄
$f_{RS̄}$
RS̄F
RS̄F̄
1:5
MORTALITY ANALYSIS
1:1
QUESTIONNAIRE
1:1
PHYSICAL EXAMINATION
CONTROL
$m_{C'}$
$1-m_{C'}$
C''
C'
C
s_C
$1-s_C$
CS
f_{CS}
CSF
CS̄F
CS̄
$f_{CS̄}$
CS̄F
CS̄F̄
UNUSED
REPLACEMENTS

Referring again to Figure 4, R and C indicate living RANCH HAND members and primary matched controls. If m_R' is the proportion of R' found to be deceased, then

$$R = (1 - m_R')R'$$

The questionnaire will provide data concerning specific symptoms and other findings in the R and C groups. Thus, various questionnaire finding rates in R, s_R, will be calculated and compared with the corresponding rates in C, s_C.

The questionnaire will allow allocation of RANCH HAND personnel into those with symptoms on questionnaire, indicated by RS, and those without, $R\bar{S}$. Similarly, the control individuals will be placed into symptomatic, indicated CS, and asymptomatic, $C\bar{S}$ groups.

The physical examination performed on individuals from R and C will allow estimation and comparison of rates of physical findings in these groups. Rates of abnormal physical findings can be symbolically indicated as f_R and f_C for RANCH HAND and control groups respectively. Comparison of these rates is very important and details will be discussed below.

Let f_{RS} be the rate of physical findings among RANCH HAND personnel with findings by questionnaire and let $f_{R\bar{S}}$ be the rate of physical findings among RANCH HAND people with no findings on their questionnaire. For most disease processes it would be expected that f_{RS} should be a larger rate than $f_{R\bar{S}}$. If f_{RS} is observed to be equal to or less than $f_{R\bar{S}}$, an interpretation of over-reporting may be warranted, although the possibility of subclinical disease is recognized. Rates f_{CS} and $f_{C\bar{S}}$ will also be estimated, and comparisons between f_{RS}, f_{CS}, $f_{R\bar{S}}$ and $f_{C\bar{S}}$ will be accomplished.

The eight rates m_R', m_C', s_R, S_C, f_{RS}, $f_{R\bar{S}}$, f_{CS}, $f_{C\bar{S}}$ and their refinements fully characterize this study. As depicted in Figure 4, "vertical comparisons of these rates provide relative risks m_R'/m_C', s_R/s_C, f_R/f_C, f_{RS}/f_{CS} and $f_{R\bar{S}}/f_{C\bar{S}}$ which are of central importance in defining herbicide effects. "Horizontal comparisons" relate f_R to s_R, f_{RS} to $f_{R\bar{S}}$, f_C to s_C and f_{CS} to $f_{C\bar{S}}$. Specifically, the ratio f_R/s_R is the ratio of physical findings to reported symptoms in the RANCH HAND population. This ratio may be contrasted with the ratio f_C/s_C and if f_R/s_R is less than f_C/s_C over-reporting is suggested. Likewise, if f_{RS} is less than $f_{R\bar{S}}$, over-reporting is further suggested. A comparison of $f_{RS}/f_{R\bar{S}}$ to $f_{CS}/f_{C\bar{S}}$ contrasts the odds of findings given symptoms in the RANCH HAND population with the odds of findings given symptoms in the control group. If these odds are lower in the RANCH HAND group, over-reporting is again implied. Further discussion of these rates is presented in Section V.G.

During the questionnaire and physical examination phases of this study, only one of the five randomly selected mortality study controls will be used for each RANCH HAND individual. If this control is unwilling to participate, another mortality study control will be used as indicated in Figures 3

and 7. These replacements will be carefully labelled for purposes of statistical analysis. A detailed discussion of this replacement concept is found in Section VI.

D. Mortality Study

(1) Introduction

The mortality, retrospective morbidity, and follow-up studies are components of a "non-concurrent" prospective study used in the observation of a specially exposed group or industrial population starting from some date in the past. The initial exposures occurred 11-19, years ago and varied in intensity and duration from one RANCH HAND member to another. Access to employment, medical, or other types of records is an obvious requisite for such a study. The classical "case-control", retrospective study is not operative in this protocol due to the lack of defined clinical endpoints. The mortality study will be conducted in two phases; a review of past mortality, and a continuing assessment of the death rate in the exposed and control cohorts over the twenty year duration of the RANCH HAND II project.

Based upon USA vital statistics, 8.6% of the study subjects are expected to have died between completion of their Vietnam tour and initiation of this study. Of these deaths in the control group, approximately 30% should have been due to cardiac causes, 24% to neoplasia, 13% to accidents, 5% to cirrhosis, and 0.1% to leukemia.

(2) Data Collection Methods

The mortality status of the exposed cohort and the randomly selected controls will be ascertained using multiple techniques including: payments of Veterans Administration Death Benefits, Social Security Administration Records, Air Force Accounting and Finance Center wage and retirement payments, and interviews with subjects or their families. Death certificates, autopsy reports, and medical records will be obtained for each deceased subject. The International Classification of Disease, Ninth Revision, 1978, will be used for coding. At the time of the first followup examination, all participants will be asked to allow an autopsy to be performed at government expense at the time of their death, and have the tissues forwarded to the Armed Forces Institute of Pathology, and the results sent to USAFSAM.

(3) Analysis of Mortality Data

(a) Basic analyses

Considering the basic groups R' and C' in Figure 4, individuals will be classified into three categories: alive, dead, or unaccounted. If a large number of individuals in each group are unaccounted for, the study can obviously be severely biased. Thus, significant effort will be expended to reduce the unaccounted category as much as possible. At most, 1 to 3 percent of both groups can be allowed to remain unaccounted, with a 1% rate

being preferred. If for example, the mortality rate in C' is 0.10, then an unaccountability rate of 0.01 could alter the mortality rate by as much as 10%. Whatever the unaccountability rates, the pattern of unaccountability must also be compared between groups R' and C'. For example, the possibility of age differences must be examined, particularly if the unaccountability rates are high. The following paragraphs discuss the analysis of mortality under the assumption that low unaccountability rates have rendered the mortality analysis meaningful.

Multiple mortality assessments will be accomplished during the course of this study, one at the beginning of the study, using available mortality data on the basic mortality cohorts in C' and R' (5:1 ratio), and others using mortality data on R' and all controls used in the study (both C' and replacements) as controls accumulate prospectively. The procedures described here will be used in all of these assessments.

Henceforth, within the protocol, the term "mortality data" does not distinguish between that data collected initially and that data collected in the future.

The mortality data will be analyzed using several different approaches. Crude age-specific death rates will first be calculated and tabulated. Age will be divided into k strata, and person-years will be observed for each strata as will be the number of deaths in each strata. In this manner a tabular display will be developed as shown in Table 7.

Table 7

STRATIFIED FORMAT OF AGE-SPECIFIC DEATH RATES

	Ranch Hand			Controls		
Age Group	Person Years	Deaths	Death Rate	Person Years	Deaths	Death Rate
1	P_{11}	m_{11}	r_{11}	P_{21}	m_{21}	r_{21}
2	P_{12}	m_{12}	r_{12}	P_{22}	m_{22}	r_{22}
3	P_{13}	m_{13}	r_{13}	P_{23}	m_{23}	r_{23}
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
k	P_{1k}	m_{1k}	r_{1k}	P_{2k}	m_{2k}	r_{2k}

Since the death rates r_{1j} and r_{2j} are Poisson variables, they can be contrasted directly. If the relationship of r_{1j} to r_{2j} is found to be consistent between age strata (within statistical variability), a summary mortality index may be calculated. One summary index that will be calculated is the Standardized Mortality Ratio (SMR) which is (Armitage, 1971):

$$SMR = M \times 100$$

$$M = \frac{\sum_{j=1}^{k} m_{1j}}{\sum_{j=1}^{k} P_{1j}\, r_{2j}}$$

"Classical" standardized mortality ratios using national mortality data as the reference will not be calculated for RANCH HAND II due to the effects of the healthy worker phenomenon. The term $\Sigma\, m_{1j}$ is the total number of deaths observed in the RANCH HAND group while $\Sigma\, P_{1j}\, r_{2j}$ is the number of deaths that would be expected were the age-specific RANCH HAND death rates the same as the age-specific control death rates. Thus the concern is for an SMR greater than 100%. If a crude death rate for controls, d_C, is calculated as

$$d_C = \frac{\sum_{j=1}^{k} P_{2j}\, r_{2j}}{\sum_{j=1}^{k} P_{2j}}$$

then the standardized crude rate for the RANCH HAND group d_{RH} is

$$d_{RH} = M d_C .$$

An approximate statistical test would regard d_{RH} as a Poisson random variable with mean d_C.

An alternative approach to the provision of a proportionate mortality ratio is that of Breslow and Day (1975). In this treatment, a multiplicative model is employed, for example:

$$\lambda_{ijk} = \theta_i \phi_j \psi_k$$

where λ_{ijk} is the mortality rate, θ_i is the contribution due to population differences (RANCH HAND versus Control), ϕ_j is the contribution due to age

group, and ψ_k is the contribution due to length of time in RVN, etc. The statistical approach here is via maximum likelihood.

Logistic models (Walker and Duncan, 1967) have been extensively studied at USAFSAM for application in cardiovascular disease analysis. These models, in the herbicide context would have the form

$$P = [1 + \exp(\alpha + \beta_1 A + \beta_2 T + \beta_3 R + \beta_4 E + \beta_5 AE + \ldots)]^{-1}$$

where

P = probability of death
A = age in years
T = length of time in RVN
R = indicator variable for race
E = exposure variable

and where α_1, β_i, i=1,2,... are coefficients to be estimated from the data. Testing for a group difference can be accomplished by estimating β_4 and the interaction coefficients such as β_5. If all interaction coefficients involving the exposure variable E are zero and E is treated as a 0/1 variable, Cox (1958a, 1958b) has shown that the most powerful test for non-zero β_4, in the setting of matched pairs, is McNemar's test. This latter test makes full use of the paired design of the study. For McNemar's test, the data are cast into a 2 x 2 table as shown in Table 8. In this table, "a" is the number of pairs in which both members have died, "b" is the number of pairs in which only the RANCH HAND person has died, etc. Using McNemar's test, the test statistic

$$\chi^2 = \frac{|b - c|^2}{b + c}$$

is calculated and referred to the chi-square distribution with one degree of freedom. Cox (1966) and Meittinen (1969) provided extensions of McNemar's test for R controls per exposed (R-to-1 matching). Of course the above analyses will be accomplished considering all deaths, and deaths by specific cause.

As previously discussed, RANCH HAND personnel may be characterized as risk takers. This risk taking behavior may be associated with increased mortality from a variety of causes. On the other hand, herbicide exposure has caused neuropathy in the RANCH HAND personnel, one could anticipate that this disability would increase the probability of accidental death. Therefore,

Table 8

FORMAT OF McNEMAR'S TEST

CONTROLS

RANCH HAND PERSONNEL	DEAD	ALIVE	TOTAL
Dead	a	b	a+b
Alive	c	d	c+d
Total	a+c	b+d	n

accidental death rates among RANCH HAND participants will be corrected for risk taking. This can be accomplished by including assessment of risk taking behavior in the questionnaire, indepth interview, and psychological evaluation. Both control and RANCH HAND mortality could be corrected using these measures, with the resultant rates being less biased and, therefore, a better indicator of exposed versus control effect.

(b) Mortality analysis without covariates.

The first step in the statistical analysis of survival data is descriptive, i.e., the construction of summary measures which provide a basis for comparing different exposure groups without any allowance for the effects of possibly confounding variables (e.g., age) except perhaps for some limited stratification. Since one must expect many "losses to follow-up", only methods which take full cognizance of this complication will be considered. It should be pointed out that all the methods described below assume independence between censoring (e.g., loss to follow-up) and death or morbid event, although some techniques permit different patterns of censoring in different exposure groups.

The life table method can be adapted to obtain a step-function approximation to survival distributions in the presence of censoring (Chiang, 1968, Gross and Clark, 1975). The failure time distribution is the function $F^o(t)$ which provides the probability of death at or before time t in the study. The Kaplan-Meier estimator of $F^o(t)$ is $\hat{F}^o(t)$ where

$$\hat{F}^o(t) = 1 - \prod_{i \in D(t)} [1 - 1/R(T_i)]$$

In this equation, D(t) is the "death set" at time t, i.e., the set of all indices i of individuals who were observed to fail before time t. $R(T_i)$ is the number of individuals who were at risk just before time T_i, the time of death (or morbid event) of the i^{th} study individual in D(t). A nonparametric approach to testing the equality of survival distributions in a matched

pair study has been developed by Wei (1980). His statistic is a generalization of the Gehan (1965a) statistic. A second test for homogeneity of survival distributions for discretized failure data is the test for marginal homogeneity in a KxK table due to Stuart (1955). Thirdly, the McCullough Model and test may be used on the KxK array to test for marginal homogeneity and stochastic ordering.

(c) Mortality analysis with covariates.

These methods allow adjustment of mortality rates or morbidity rates using covariates such as age, race, length of time in RVN, AFSC, risk taking score, etc. For the purposes of this discussion it will be assumed that the covariables are categorical, that there are only two such covariables and the covariables do not interact in affecting the hazard of death or morbidity. These assumptions can all be relaxed using available methods.

The hazard function $h_i(t)$ for the ith individual in the study is the function which provides the conditional probability of death or morbid event in the time interval $(t, t+dt)$ given his survival up to time t. The function $H_i(t)$ where

$$H_i(t) = \int_0^t h_i(\tau)d\tau$$

is called the cumulative hazard for the ith individual. It is readily shown that the failure time distribution $F_i^o(t)$ is given by:

$$F_i^o(t) = 1 - \exp(-H_i(t))$$

From this last equation it follows that h_i and F^o are transforms of each other, hence the dependence of F^o on covariables may be modeled via h_i. This may be accomplished as follows. Let $X_i(t)$ and $Y_i(t)$ denote discrete valued stochastic processes pertaining to the ith individual and describing two covariates of interest (e.g., one may be an exposure variable and the other may be covariate such as age or crew position). A basic model for hazard is:

$$h_i(t) = \exp\left[\xi X_i(t) + \eta Y_i(t)\right]$$

where ξ and η are "log-relative risks". This model may be extended to allow for any number of possibly interacting factors. Inference about log-relative risks may be drawn using either an approach derived from D. R. Cox (1972) by E. Peritz and R. Ray (1978) or using an approach described by Frank (1977). Another model, termed the proportional hazards model, is given by

$$h_i(t) = \lambda_0(t)\exp\ [\beta X_i(t)]$$

The proportional hazards model has been discussed, for the special case that Xi(t) does not change with time, by Cox (1972). A test for the equality of survival distributions in a matched pair study which incorporates the proportional hazard model has been given by Breslow (1975). A test of fit for the proportional hazards model is given by Schoenfeld (1980).

E. Morbidity Study

(1) General Considerations

A vigorous attempt to determine the morbidity experience of all exposed subjects and their primary controls will be undertaken using questionnaires, indepth personal interviews, and physical examinations. A waiver will be requested from the U.S. Attorney General so that medical information collected during the conduct of this study may be exempted from subpoena into Federal Court. Total confidentiality of medical information will be granted to subjects who are not on active duty, and partial confidentiality will be given to active duty subjects with release of information to the DOD only in instances where there is a public safety or national security risk. The schedule and method of contact with the study subjects is depicted in the Appendix Table A-7.

(2) Questionnaire Methods

All living exposed subjects and their primary controls will be offered a comprehensive personal and family health questionnaire administered in the subject's home by a civilian contractor.

In addition to subject interviews, a face-to-face interview will be conducted with the current spouses of the subjects to obtain a more accurate and complete assessment of fertility and reproductive function. Reproductive information that will be collected includes but is not limited to the number of live births, the number of still births, the number of miscarriages, the number of children conceived, the number of abnormal offspring, and the total years of marriage. Previous spouses of divorced or remarried subjects will also be interviewed to obtain similar data. Interviews with the first order next-of-kin of deceased subjects will provide morbidity data on the subject prior to his death. Whenever subjects, their spouses or next-of-kin will not consent to participate in a face-to-face interview, attempts will be made to elicit the information by telephone.

The questionnaire is an important part of this study because noncompliance rates for the physical examination and its face-to-face medical interview are expected to be substantially greater than non-compliance with the initial questionnaire. The questionnaire serves a four-fold purpose: (1) to capture baseline personal and medical data on subjects who might be noncompliant for subsequent physical examinations, (2) to serve as a cross-reference

source for objective data obtained at the time of physical examination, (3) to obtain a targeted medical inventory, independent of the physical examination process, and (4) to obtain health perception data to serve as a foundation for the replacement strategy. As depicted in the Appendix, Figure A-2, only an estimated 40% of the RANCH HAND population will participate in the examination, while at least 65% will respond to the questionnaire. The information collected by questionnaire from these additional 309 individuals and their controls will provide valuable morbidity data which would otherwise be lost. The questionnaire (see Section XI) will emphasize identification data, RVN tour history, dermatologic conditions, neuropsychiatric conditions, fertility aberrations, genetic defects in offspring, sensory defects, and personality factors. A targeted medical inventory will be included in the questionnaire, and will inventory symptoms prior to, during, and after duty in RVN as well as those currently manifested. It will take approximately six months to complete all initial questionnaires on both groups. The questionnaire will be "field-tested" by the contractor on former Air Force personnel with RVN experience. Specific questions on the questionnaire will be directed to verifiable information, wherever possible. Questionnaire development and refinement, including specific response verification procedures have been pursued through civilian contract. Questionnaire data will be cross-linked and integrated with medical record information and physical examination findings. Questionnaire data from individuals not completing all phases of the study will not be discarded, but will be incorporated within the entire data base where statistically appropriate. Each participant will be asked to sign release forms so that all civilian health records, including those of dependents, can be obtained and reviewed as necessary. Attempts will be made to obtain pathological reports and specimens following surgical procedures. Federal health records on all family members on file in the NPRC will be retrieved. For retired members, and separated members with VA privileges, all available VA medical records will be obtained. All retrieved medical records will be reviewed, scored, compared to questionnaire data for reliability, and then be entered into a repository system. Identified participants who are non-responsive to questionnaire will be pursued to determine status, disinterest, moribund state or death, etc. These individuals will be cross-referenced in other federal record systems in an attempt to achieve total ascertainment. Death certificates and autopsy reports will be retrieved on all dead exposed and matched control subjects for the mortality analysis. Birth/death certificates will be sought for all offspring.

(3) Physical Examination

A voluntary comprehensive physical examination will be offered to all individuals in both the exposed and primary control groups within one year of questionnaire administration. The condition for entry into the examination phase of the study will be the completion of the baseline questionnaire. In the event that the primary control does not complete both the questionnaire and the physical examination, a replacement will be selected from the control set [See Figure 3 and Section F(3)]. Statistical testing will be conducted by a variety of techniques on both questionnaire and examination findings. At the time of physical examination, an extensive physical examination, medical

history, and review of symptoms will be conducted. A standardized protocol will be used to insure comparability of data. This will provide cross-reference data to the initial questionnaire and to medical record data, if retrievable. Specific response verification and bias indicator questions will be included in this interview as well.

(a) Examination Parameters

A comprehensive physical examination will be conducted on all willing participants. The examination will be structured as outlined below and in Section XII and will be performed at the earliest practical time following the completion of the questionnaire. The close sequencing of these study components will limit the development of major symptoms in the interval between the questionnaire and the examination. Examinations will be performed under contract at a single civilian medical center having dermatologic, neurologic and electromyogram/ nerve conduction capabilities. Informed consent forms will be obtained for all procedures. Physicians and technicians will handle all participants without a knowledge of exposed or control status, and will conduct the examinations by standardized protocols to minimize variability. Medical students, interns, and residents will not be allowed to perform these examinations, and specialty trained neurologists and dermatologists will perform the appropriate portions of the examination. An onsite monitor will insure that the examination protocol is followed. All laboratory tests will be subject to rigid quality control. Laboratory and physical examination data will be measured on a continuous scale whenever possible in order to improve statistical power in the analysis.

Under special circumstances, additional testing will be accomplished. Karyotyping of the individual and his family members will be considered if clinical history or physical examination findings are suggestive of this need. Most well conducted studies have shown that, when present, chromosomal abnormalities due to TCDD are transient. If on detailed analysis of the baseline examination and questionnaire, reproductive areas are heavily affected, routine karyotyping may be included in the test battery for the followup phases of the study. TCDD analysis on blood and urine will be considered in the future provided that (1) strong cause and effect relationships can be ascribed to Herbicide Orange and (2) high resolution mass spectrometry technology achieves 10 femtogram sensitivity with high isomeric specificity. Serum, urine, and semen specimens will be obtained from all participants, aliquoted, and preserved at -70°C for possible analysis in the future. These serum and/or urine specimens will also be used for analysis of porphyrin metabolites if analytic techniques make this a feasible diagnositc procedure. Extensive immunologic function analyses will be conducted on a randomly selected group of subjects.

Physical examination and laboratory data will be placed in the member's coded master file for detailed cross-analysis to questionnaire data. Information identifiable to the subject will not be released without his consent in accordance with the Privacy Act. However, in accordance with Air Force regulations, active duty flying personnel and active duty air traffic controllers found to have conditions which are disqualifying for flying duty will be temporarily "grounded" pending resolution of the medical condition.

Physical Examination Profile

General Physical Examination	Hemoglobin	CPK
FBS, 2 Hr Post Prandial	Hematocrit	ECG
Urinalysis	White Blood Cell Count and Differential	Chest X-Ray
BUN/Creatinine	Platelet Count	VDRL/FTA
Cholesterol/HDL	RBC Indices	Cortisol Differential
Triglycerides	Sedimentation Rate	Thyroid Profile (RIA)
Serum Protein Electrophoresis	Prothrombin Time	Pulmonary Function Studies
		Blood Alcohol

Dermatologic Examination
- Urine Porphyrins
- Urine Porphobilinogen
- Delta-aminolevulenic Acid

Neuro-Psychiatric Examination
- General Neurologic Examination
- Psychological Battery:
  - MMPI
  - WAIS
  - WRAT
  - Halstead-Reitan
  - Wechsler Memory Scale Subtests
  - Cornell Index
- Nerve Conduction Velocities

Reproductive Examination
- LH, FSH, Testosterone
- Semen Analysis

Neoplastic/Hepatic Examination

SGOT	Alkaline Phosphatase
SGPT	LDH (Isoenzymes if elevated)
GGTP	Hepatitis B Antigens/Antibodies
	Bilirubin, Total and Direct

Additional Studies (Individuals with abnormal history or examination)

Karyotyping	Immunoelectrophoresis
Hepatitis A Antigens/ Antibodies	Bilateral profile and full-face photographs
Anti-Nuclear Antibody	Skin Biopsy
Quantitative Immunoglobulins	Additional Consultations as Required

Immunologic studies (conducted on a randomly selected group of subjects)
- Enumeration of B and T Cells
- Enumeration of Monocytes
- B and T Cell Function

(4) Analysis of Questionnaire and Physical Examination Data

The Questionnaire and Physical Examination will produce data of three types: (1) dichotomous, (2) polytomous and (3) continuous.

Dichotomous (e.g., present/absent) rates will be evaluated using the tools described above for mortality analysis. For example, the questionnaire will provide data concerning the first occurrence of disease states by age, and standardized rates and relative risks may be calculated. The occurrence of such findings can be related to age, time spent in RVN, exposure, and other variables using logistic models followed by McNemar's test where appropriate. These tests will examine the presence or absence of group effect and allow assessment of the statistical significance on non-unity relative risks.

Polytomous findings will occur in both questionnaire and physical examination responses. As an example consider retinal findings categorized into four grades, and studied as a function of age and exposure group as represented in Table 9. In this table the x_{ijk}'s are counts of occurrence. In analyzing tables such as these, techniques as described by Bishop, Fienberg, and Holland (1975) will be used. Specifically, if m_{ijk} is the expected value of x_{ijk}, general log-linear models of the form

$$\ell n\, m_{ijk} = u + u_1(i) + u_2(j) + u_3(k) + u_{12}(ij)$$

$$+ u_{13}(ik) + u_{23}(jk) + u_{123}(ijk)$$

will be used, where $u_1(i)$ is the effect of RANCH HAND membership alone on cell frequency, $u_{12}(ij)$ is the effect of an interaction on RANCH HAND membership with retinal grade, etc. This model can work with dichotomous as well as polytomous data. Under appropriate conditions on expected values of entries in Table 9, the pairing in the study design can be used with the data being organized as shown in Table 10. In Table 10, N_{ij} is the number of pairs such that the exposed person has retinal grade i, and the control person has retinal grade j. Appropriate tests for this setting are indicated by Fleiss (1973) and McCullough (1978).

With regard to continuous variables, the intended method follows Carpenter (1977) who found substantial gains in analysis efficiency by matching cases, subsequently employing covariance analysis to remove non-controlled effects. The conditional logistic regression model for relative risk, Holford, White and Kelsey (1978), is also applicable and will be used.

Table 9

FORMAT OF CATEGORICAL REPRESENTATION OF RETINAL CHANGES

Retinal Category \ Age Category	RANCH HAND PERSONNEL				CONTROLS			
	1	2	3	4	1	2	3	4
1	X_{111}	X_{112}	X_{113}	X_{114}	X_{211}	X_{212}	X_{213}	X_{214}
2	X_{121}	X_{122}	X_{123}	X_{124}	X_{221}	X_{222}	X_{223}	X_{224}
3	X_{131}	X_{132}	X_{133}	X_{134}	X_{231}	X_{232}	X_{233}	X_{234}
4	X_{141}	X_{142}	X_{143}	X_{144}	X_{241}	X_{242}	X_{243}	X_{244}

Table 10

FORMAT OF PAIRING FOR GRADES OF RETINAL FINDINGS

RANCH HAND Grade \ Control Grade	1	2	3	4
1	N_{11}	N_{12}	N_{13}	N_{14}
2	N_{21}	N_{22}	N_{23}	N_{24}
3	N_{31}	N_{32}	N_{33}	N_{34}
4	N_{41}	N_{42}	N_{43}	N_{44}

(5) Analysis of Fertility/Reproduction Data. The herbicides under consideration in this study have been alleged to effect fertility and/or reproductive functioning. An attempt will be made to address these allegations by analyzing at least three primary variables: the total number of conceptions since exposure in RVN, the number of miscarriages in spouses since exposure in RVN and, the number of abnormal offspring since exposure in RVN. The interview with current and former spouses will provide much more accurate information on fertility and reproductive functioning than if similar data were obtained from the male subjects themselves. The study questionnaire will provide the numbers of miscarriages, abnormal offspring and of live births. The sum of the number of miscarriages, still births, and live births will provide an estimate of the total number of conceptions. If differing divorce rates are found in the RANCH HAND and control groups, this may render the average number of years of marriage and the distribution of the years of marriage different in the two groups. This will be investigated and adjusted

for if need be, either by analyzing total number of conceptions divided by (or normalized by) the number of years of marriage, or by using a more detailed covariance analysis. Further, the ratio of the number of miscarriages to adjusted total conceptions will be calculated and compared, as will be the ratio of the number of abnormal births and adjusted total conceptions.

In summary, the following statistics relating to fertility will be calculated and analyzed at the very least:

$$\text{TOTAL CONCEPTIONS} = \#\text{Live Births} + \#\text{Still Births} + \#\text{Miscarriages}$$

$$\text{NORMALIZED FERTILITY INDEX} = \frac{\text{TOTAL CONCEPTIONS}}{\text{YEARS OF MARRIAGE}}$$

$$\text{MISCARRIAGE FRACTION} = \frac{\#\text{ MISCARRIAGES}}{\text{TOTAL CONCEPTIONS}}$$

$$\text{ABNORMALITY FRACTION} = \frac{\#\text{ ABNORMAL OFFSPRING}}{\text{TOTAL CONCEPTIONS}}$$

F. Follow-up Study

(1) Study Adaptations

Following complete data analysis of the initial mortality and morbidity studies, adaptive or restrictive health surveys will be developed and administered to all follow-up study subjects three, five, ten, fifteen and twenty years after the initial questionnaire. Similarly, a condensed physical examination profile that will achieve adequate sensitivity and specificity for prospective diagnosis will be developed. The adaptive physical examination will be offered to all follow-up participants, and will also be conducted in years three, five, ten, fifteen, and twenty (see Appendix, Table A-5). An interim examination in year three is essential in this study because the age group under study is approaching that portion of the mortality/illness incidence curve with the steepest slope. A lapse of five years between the first two examinations could easily miss significant development of disease in the intervening years. Ample precedent for interim examinations can be found in the Framingham cardiovascular disease study, and in the follow-up evaluation of West Point graduates being conducted by the Air Force.

(2) Entry Criteria

All exposed or control individuals completing the baseline questionnaire and physical examination will be entered into the follow-up; further continuation will depend upon the member's willingness/ability to participate in additional health surveys and condensed examinations. Specific study entry rules are detailed in Table A-6 and Figure A-3 of the Appendix.

(3) Loss to Study; Key Issues

Loss of participants over time adversely affects any epidemiologic study in two ways. As the sizes of the study groups decrease, statistical power also declines, and bias is injected into the study if losses are not randomly distributed in the study populations. It is reasonable to assume that in this study, losses will be non-random with greater non-compliance among individuals who perceive their health as "well," since there is less incentive for this group to continue participation. As shown in Figure 5, such a differential pattern of loss will alter the population, and skew the frequency distribution curve.

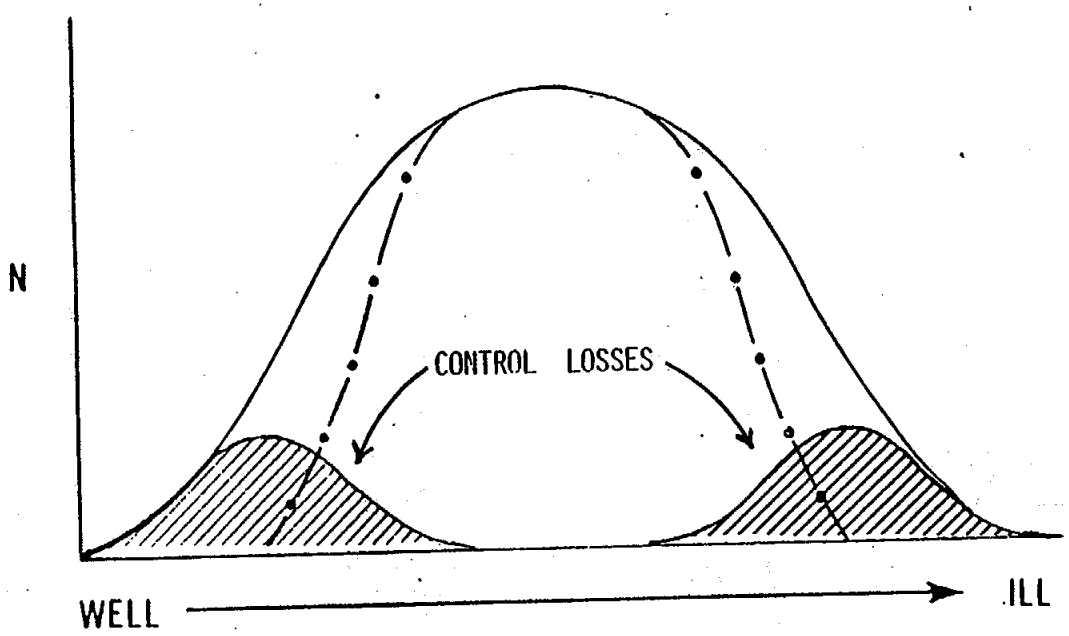
Most previous epidemiologic studies have approached the problem of declining statistical power by beginning the study with multiple controls per exposed subject, and passively allowing attrition to occur throughout the study period. However, this approach does not address the problem of bias. This study will take an active approach to both of these problems by using a replacement concept. As a control is lost to study, a replacement will be chosen from the original set of ten matched controls. The replacement will be selected from the control set, and will have a perception of health similar to that of the lost control (Figure 6). The replacement strategy will maintain statistical power and the integrity of the matched design despite loss to study in the control group, and will correct anticipated bias while minimizing the number of required physical examinations.

At the initiation of the follow-up study, loss of an exposed member will not be cause to cease surveillance of his primary matched control. In the event of a control loss (for reasons other than death), another control from the set will be brought to study (Figure 7), the comprehensive questionnaire will be administered, and a baseline physical examination performed.

If a control is noncompliant for one portion of the study and is replaced by another control, the noncompliant individual will be approached at the time of subsequent questionnaires and examinations, and encouraged to reenter the study. If he reenters, both he and the replacement will be included in the evaluation. Similarly, noncompliant exposed subjects will also be aggressively recruited for all subsequent study phases.

FIGURE 5

EFFECT OF NON-RANDOM LOSS TO STUDY IN THE CONTROL POPULATION



- IF CONTROL LOSSES ARE ILL, A SPURIOUS EFFECT IS ATTRIBUTED TO HERBICIDE EXPOSURE.
- IF CONTROL LOSSES ARE WELL, A TRUE/VALID HEALTH EFFECT IS DILUTED.

FIGURE 6

THE REPLACEMENT STRATEGY

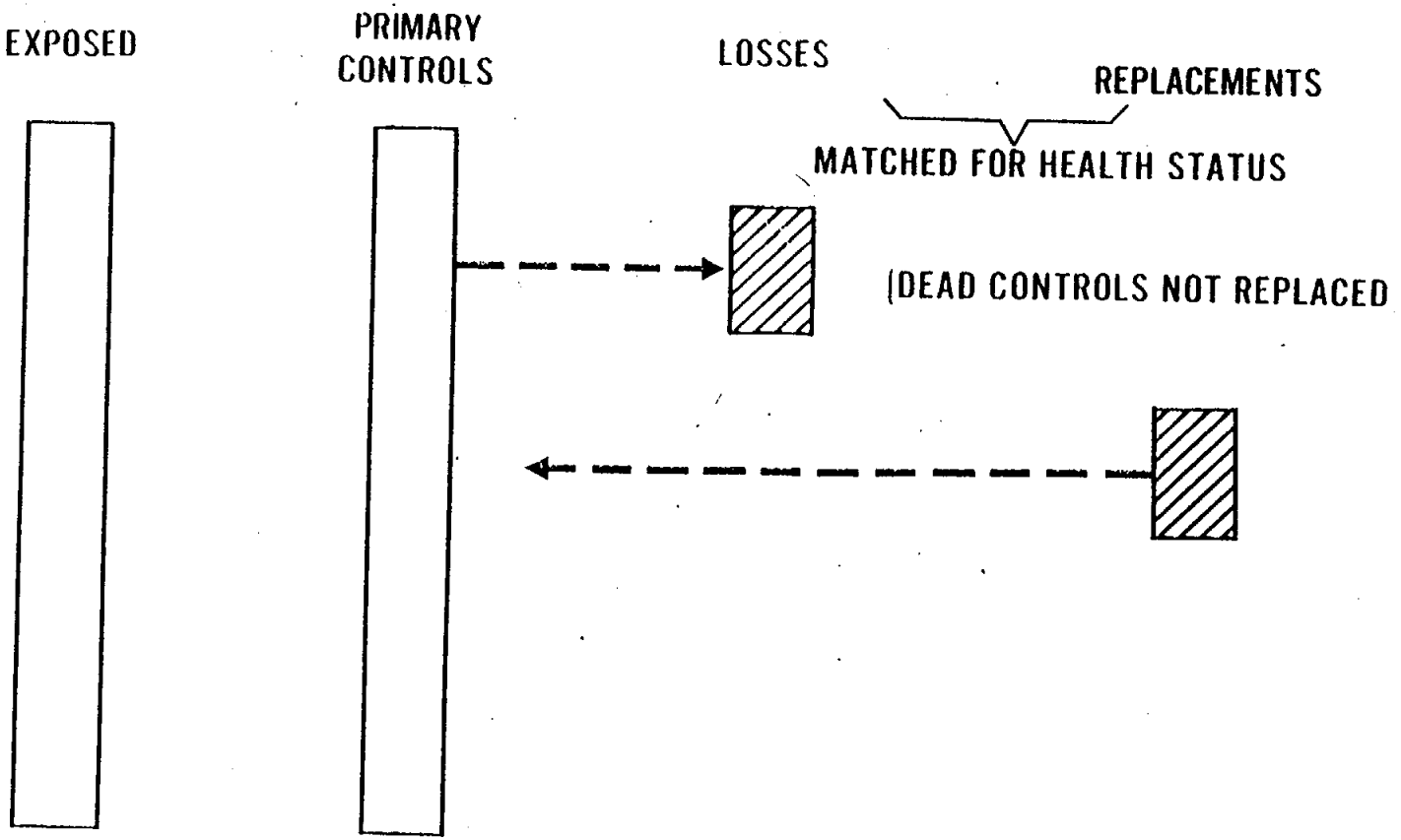
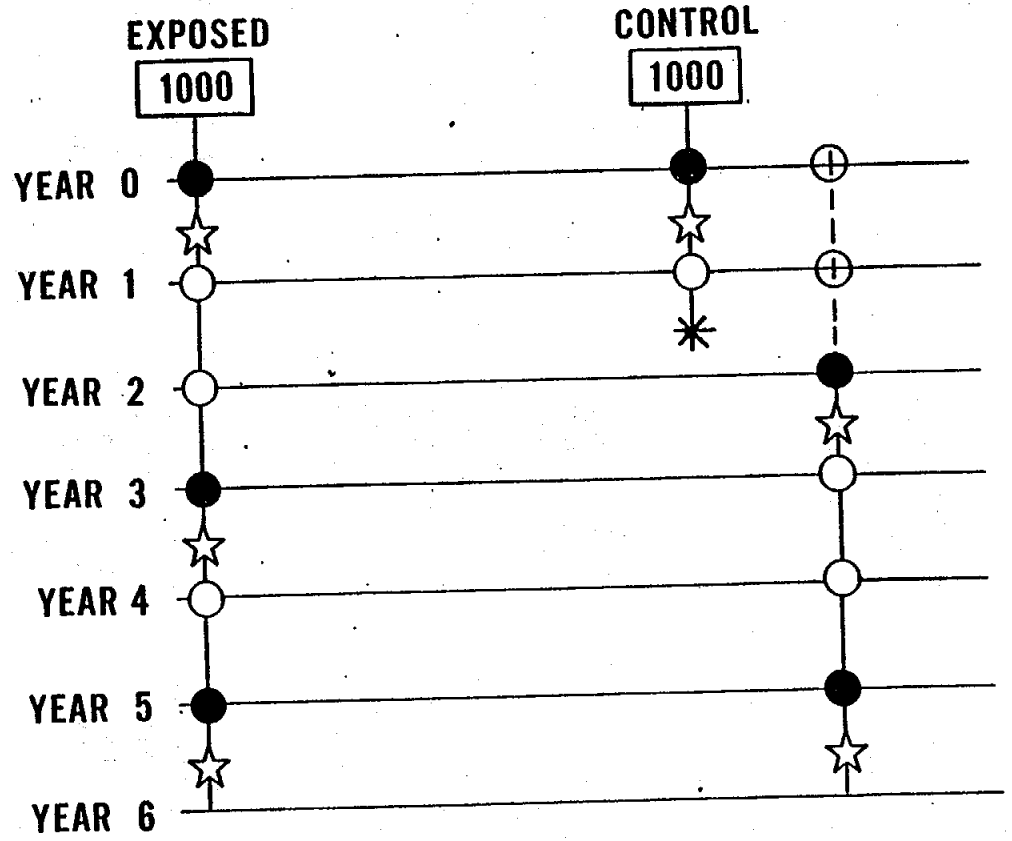


FIGURE 7

CONTROL REPLACEMENT FOR THE MORBIDITY AND FOLLOW UP STUDIES



For exposed and control individuals who drop out of the study but subsequently re-enter, medical data for the intervening years will be reconstructed from questionnaire and interview responses. IN ALL CASES OF LOSS-TO-STUDY, INTENSIVE EFFORTS WILL BE MADE TO DETERMINE THE SPECIFIC REASONS FOR NON-COMPLIANCE, AND DATA FROM REPLACEMENT CONTROLS WILL BE REVIEWED TO ASSESS COMPARABILITY WITH THE LOST INDIVIDUALS. Medical record reviews of new entrants will continue throughout the follow-up period.

(4) Study Length

The follow-up study is initially planned for 20 consecutive years. Procedures, progress, and interim results of the study will be monitored by an independent scientific review group, responsible to the Office of Science and Technology Policy in the White House.

G. Determination of "Disease"

(1) Introduction

Since this study is dealing with an unknown clinical endpoint with unknown latency, determination of a disease state by statistical methodology is a prime scientific thrust of the investigation. From the literature, chloracne is the only generally accepted chronic disease associated with high exposure to dioxin. The questions of primary interest are: (1) Does a history of chloracne invariably lead to future disease? and (2) In the absence of chloracne, is there emergence of other attributable diseases? Under a broad concept of "spectrum of illness", either or both of these conditions are possible. The clarification of their respective contributions to the natural history of past or of subsequent "disease" is of significant interest.

(2) Discussion

Inferences about a disease state from this study can be derived from several logical approaches. These approaches can be grouped into two categories: (1) those dealing with symptoms which can be used to construct a symptom complex that may represent disease, and (2) those dealing with physical signs which in themselves represent disease. In the former, one can form a subset of individuals that have symptoms (e.g., infertility) and study them during the morbidity and follow-up studies. Focusing on the overall patterns of alleged symptoms and categorizing them into a symptom complex may identify those individuals with a disease syndrome, or those at higher risk of developing disease (e.g., genetic disorders, cancer). In the latter approach, data on abnormal physical signs (e.g., genetic defects in offspring) and laboratory results can be compared between exposed and non-exposed groups in an attempt to again establish the presence or absence of disease. By putting this array of data into a logical decision-making scheme, specific relative risks can be calculated in the follow-up study, and specific response patterns can be inferred as shown in Figure 8.

FIGURE 8

INTERPRETATION OF HORIZONTAL COMPARISONS

OVERT EFFECT	SUBCLINICAL	OVER-REPORTING
$M_R \succ M_C$	$M_R = M_C$	$M_R = M_C$
$S_R \succ S_C$	$S_R = S_C$	$S_R \succ S_C$
$F_R \succ F_C$	$F_R \succ F_C$	$F_R = F_C$
$F_{RS} \succ F_{CS}$	$F_{RS} \succ F_{CS}$	$F_{RS} \prec F_{CS}$
$F_{R\bar{S}} \succ F_{C\bar{S}}$	$F_{R\bar{S}} \succeq F_{C\bar{S}}$	$F_{R\bar{S}} = F_{C\bar{S}}$
MORTALITY/SYMPTOM/ SIGN REGRESSION ON EXPOSURE	SIGN REGRESSION ON EXPOSURE	NO REGRESSION ON EXPOSURE SEEN

$$F_R = F_{RS}\, S_R + F_{R\bar{S}}\, (1 - S_R)$$

Again referring to Figure 8, at least three clinical patterns can defined. These patterns are delineated using relative risks (mr/mc, sr/sc, fr/fc etc., between group or "vertical" comparisons, referencing Figure 4) and using within group ("horizontal" study) comparisons such as regressing symtoms and findings rates against an index of herbicide exposure, and other comparisons. Specifically, an overt clinical effect would be marked by: an increased mortality rate in the RANCH HAND group (mr > mc), an increased rate of symptom formation in the RANCH HAND group (sr > sc), and an increased rate of objective medical findings in the RANCH HAND group as compared to the control group (fr > fc). Further, the occurrence of physical or objective medical findings would consistently relate to symptoms in the overt case (that is, frs > fcs and $fr\bar{s} > fc\bar{s}$), and finally, in the classic instance, mortality, and symptom and sign formation would be seen to be increased with increasing herbicide exposure.

A subclinical pattern is indicated in the central column of Figure 8. In this setting, one expects no statistically significant differences in mortality or symptom reporting between the two groups, exposed versus control. However, one expects a consistent predominance of medical signs in the RANCH HAND group with regression of the signs on increasing herbicide exposure.

A pattern strongly suggesting over-reporting is presented as the right column of Figure 8. In this setting, there is no difference between the groups as regards mortality or medical sign incidence; however, more symptoms are reported by the RANCH HAND group. While in this pattern the RANCH HAND subjects are reporting more symptoms, objective medical finding rates are not consistent with symptom reporting. When no regression of symptoms on exposure level is found, over-reporting is clearly and strongly suggested.

This discussion of response patterns has used regression on an exposure index in a central way. Development of such an index is discussed below. It is noted, however, that a direct index of exposure can be confounded by other factors such as cellular repair mechanisms or bioaccumulation in adipose tissue with release over time upon weight loss. Use of other factors, such as time since exposure, should help to overcome these confounders.

The strength of any inferences made from these analyses is dependent upon the statistical power inherent in the study. In addition, due to the possibility of latency being a factor in this study, a negative analysis at any time within the study does not categorically imply lack of disease, since sufficient time for emergence may not have passed.

H. Exposure Indices

(1) Exposure Concepts

A major concern in conducting this study is the lack of accurate exposure data. Although most personnel assigned to RANCH HAND squadrons were undoubtedly exposed to Herbicide Orange and TCDD, the exposures within the

group must have varied widely. Exposure to herbicides and TCDD by RANCH HAND personnel occurred almost daily. Anecdotal information suggests that many had direct skin contact which was repetitive over a long period of time (one-year tour for most individuals). Further, it is also suggested that most RANCH HAND personnel felt that the herbicides employed in the operations were not toxic to animals and man, and hence, they did not exercise the caution in handling these chemicals that is recommended today.

From a historical review of RANCH HAND operations, it appears most individuals can be classified into one of three groups based on their likely potential for exposure to the herbicides:

(1)	Pilots, Co-pilots and Navigators:	low potential
(2)	Crew Chiefs, Aircraft Mechanic, and other Support Personnel:	moderate potential
(3)	Console Operators and Flight Engineers:	high potential

The "pilot" group received most of their exposure during preflight checks as well as during the actual dissemination missions. The crew chief group experienced contact with herbicides during dedrumming and aircraft loading operations, as well as during on-site repair of the aircraft and spray equipment. The console operator group was exposed while supervising the loading of the aircraft, during ground testing of equipment, and by tank leakage during dissemination missions.

The available historical records on Operation RANCH HAND indicate that personnel assigned to the project seldom had a "routine" work schedule or environment, thus complicating estimates of the level of herbicide and dioxin exposure. Since actual exposure data (e.g., mg of herbicide/kg body wt) are not available, an exposure index will be used. The exposure indices will be calculated for each RANCH HAND individual to obtain frequency distribution, and will be calculated by evaluating the known factors that would have influenced exposure. These will include such factors as:

(1) Date of tour with RANCH HAND in Vietnam.
(2) Number and lengths of tours in Vietnam with RANCH HAND.
(3) Number of herbicide dissemination missions (as reflected by flying hours and air medals).
(4) Herbicides employed (records are available that reflect the amount of each herbicide sprayed each month and year).
(5) Crew position.
(6) Routes of exposure (the major route of exposure for most RANCH HAND personnel was probably percutaneous, although exposure through inhalation may have also been significant).

A crude exposure index which is applicable to the entire RANCH HAND cohort is expressed with the following formula:

$$E_i = q_i \times T_i$$

In this formula, E_i is the calculated exposure for the i^{th} RANCH HAND member, q_i is the quantity of TCDD-containing herbicide sprayed from aircraft assigned to the i^{th} subject's base during his assignment, and T_i is the length of the i^{th} subject's assignment (tour length). However, great care must be exercised when applying the above index. For example, the index should be used as an independent regression variable against clinical findings only within occupational strata, to avoid confounding occupational effects with exposure effects. Different degrees of regression between clinical findings and the exposure index can be expected in differing occupational groups since: (a) modes of exposure are likely to be different in different occupational categories, (b) socioeconomic correlates within occupational category could confound an herbicide effect, and (c) other exposures which could synergistically or antagonistically interact with TCDD-containing herbicide may be correlated with occupational category.

Another factor which must be considered when applying this crude exposure index is the problem on confounding a possible herbicide effect with an effect associated with tour length. Being in a comabt zone is a major psychophysiological stress, and time spent in such an area may be significantly associated with changes in long term morbidity and/or mortality. This crude exposure index, when used alone, could result in a positive regression with disease incidence or prevalence which is not due to the herbicide exposure. An approach that will correct for this potential confounding is to regress observed medical findings on both E_i and T_i to differentiate the independent effects of herbicide exposure and combat zone experience.

The values of q_i and T_i needed to calculate E_i are generally available from government records. Specifically, tour dates are available from military personnel records, and the quantity of herbicide sprayed is available for the period January 1965 through April 1970 from the "HERBS TAPES." These tapes are comprised of computerized data obtained from actual spray mission reports. This material provides the date, base of mission origin, amount and type of material sprayed (Herbicides Orange, Blue, or White) and location of the intended spray target. Estimates of the amount of herbicide sprayed prior to 1965 may be available from procurement records for Herbicides Purple, Pink, and Green, which were sprayed exclusively from Tan San Nhut Air Base from 1962 through 1964.

Animal data imply that TCDD is the most toxic component in the herbicides used in RVN. By using q_i, the amount of herbicide sprayed, one is using a variable that roughly correlates with TCDD exposure. However, it would be highly desirable to be able to analyze observed health effects in terms of specific TCDD exposure. The material sprayed from 1965-1970 had significantly lower

TCDD contamination then did those herbicides manufactured and purchased prior to 1962 and used from 1962 through 1964, but due to data limitations from a scarcity of Herbicide Purple, Pink, and Green samples, TCDD concentration profiles for those chemicals cannot be quantitatively determined. However, it may be feasible to develop estimates of the degree of contamination based upon the TCDD concentration from military and manufacturers' data.

As another approach to examining the effect of TCDD itself, one might consider stratifying the exposed cohort by date of assignment in Vietnam, expecting that those assigned earlier were more heavily exposed to TCDD. While it may well be true that earlier assignees were exposed to higher TCDD concentrations, it is unlikely that differences between "early" and "late" assignees, if they occur, can be reliably attributed to TCDD concentration changes, since several potentially confounding variables exist: (a) volunteerism among early assignees, (b) differing assignment patterns between early and late RANCH HANDers (TDY vs long term pattern) and (c) different RVN living conditions.

It is preferable to use an exposure index which is more closely tailored to the specific individual than the crude index discussed above. While T_i is subject specific, q_i is a value which refers to all individuals on the base during the period of time represented by T_i. A refined index for ground crew can be expressed as:

$$E_i = F_i \times q_i \times C \times T_i$$

where,

F_i = Average flights per day served by the i^{th} ground crew member.
q_i = Average quantity of herbicide dispensed by flights served by the i^{th} ground crew member.
C = Estimated TCDD concentration of the herbicides in use during the i^{th} subject's tour of duty.
T_i = Time spent in TVN in days for the i^{th} ground crew member.

The variable F_i can be estimated by dividing the number of RANCH HAND flights per day by the number of crew chiefs during the time period T_i. All other variables are estimated as with the crude index.

A refined index is also possible for aircrew members and is expressed as follows:

$$E_i = M_i \times D_i \times q_i \times C \times P_i$$

where,

M_i = total number of missions flown by the i^{th} air crew member.
D_i = average duration of missions flown by the i^{th} air crew member.
q_i = average quantity of herbicide dispensed per flight served by the i^{th} air crew member.
C = estimated TCDD concentration of the herbicides in use.
P_i = a crew position weighting factor.

As with the refined ground crew index, this refined aircrew index cannot be directly calculated in a strictly quantitative sense using available government records, since records to specifically link missions with particular individuals are not available to objectively determine M_i and D_i. However, reasonably accurate estimates of these parameters may be feasible using questionnaire data. Also air medal awards may allow an indirect estimate of M_i.

The crew position parameter P_i must also rely upon estimations. While the specific crew duties of each subject are known, the differential exposures associated with the crew positions within the C-123 aircraft were not determined during RVN spray missions. The 355th TAS/Spray Branch, Rickenbacher AFB OH is presently using the C-123 aircraft, configured with the A/A 45 Y-1 Internal Dispenser and attempts to assess P_i can be made. Air flow measurement and herbicide simulant deposition studies conducted by Meek are performed during the course of four C-123 flights. However, difficulties with the measurement equipment limit the validity of the value of the data in an exposure index. Further work along these lines could yield a more quantitative position weighting factor, P_i, for each individual.

Refined ground crew and air crew exposure indices can be used singly or in combination with the crude exposure index first presented; however, as with the crude index, confounding must be avoided when the refined indices are used in statistical analyses.

The exposure indices listed above are, of course, only applicable to the Ranch Hand cohort. As mentioned, a positive regression of disease incidence or prevalence with increasing exposure index will strongly support herbicide causation. We do not wish to minimize however the role of RANCH HAND versus control group disease incidence/prevalence differences as indicators of a herbicide effect. A major component differentiating the RANCH HANDers from the controls is the increased residence of RANCH HANDers in the RVN itself. If within country time does not correlate with disease incidence, RANCH HAND versus control disease incidence differences may be strongly related to herbicide. If in-country time is significant as a disease correlate, this in itself will be valuable information with regard to assessment of the RVN experience.